Cardiac dysfunction induced by experimental myocardial infarction impairs the host defense response to bacterial infection in mice because of reduced phagocytosis of Kupffer cells

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Objective: This study was undertaken to investigate the effects of cardiac dysfunction induced by experimental myocardial infarction on the host defense response to bacterial infection and the role of Kupffer cells in mediating this response.

Methods: Myocardial infarction was induced in C57BL/6 mice by ligation of the left anterior descending coronary artery. Mice were challenged with *Escherichia coli* intravenously 1, 5, and 14 days after myocardial infarction or sham operation. Thereafter, the cytokine production and the function of their Kupffer cells were assessed.

Results: Mice with myocardial infarction showed remarkable cardiac dysfunction and had a significantly lower survival than sham mice after bacterial challenge at 5 days after surgery; bacterial challenge at 1 or 14 days after surgery resulted in no difference in survival between myocardial infarction and sham mice. The phagocytic activity of Kupffer cells, assessed by fluorescein isothiocyanate microspheres, remarkably decreased in mice with myocardial infarction 5 days after surgery. Serum peaks of tumor necrosis factor and interferon-γ after bacterial challenge were also suppressed in mice with myocardial infarction at 5 days. Production of these cytokines and immunoglobulin-M from liver mononuclear cells was also impaired in mice with myocardial infarction. Enhancement of the phagocytic activity of Kupffer cells by C-reactive protein significantly improved survival after infection in mice with myocardial infarction, although neither interleukin-18 nor immunoglobulin-M treatment improved survival.

Conclusions: Cardiac dysfunction induced by myocardial infarction renders mice susceptible to bacterial infection and increases mortality because of a reduced ability of Kupffer cells to clear infectious bacteria. C-reactive protein-enhanced phagocytic activity of Kupffer cells may improve the poor prognosis after bacterial infection in mice with myocardial infarction. (J Thorac Cardiovasc Surg 2010;140:624-32)

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Supplemental material is available online.

Continued advances in cardiac surgery, such as improvements in myocardial protection, surgical techniques, and perioperative care, have extended surgical indications for patients with severe left ventricular (LV) dysfunction.[1] However, patients with low ejection fraction (<20%) still have a higher incidence of perioperative complications, such as respiratory failure, renal failure, or bacterial infections, compared with those with greater ejection fraction (>40%).[2] In particular, patients who have congestive heart failure are susceptible to bacterial infection after cardiac surgery, leading to a poor prognosis.[3] Although the incidence of perioperative infection in cardiac surgery ranges from 2.7% to 9%,[3-5] patients who have perioperative infections, including mediastinitis, thoracotomy or vein harvest site infection, or septicemia, have significantly higher mortality and length of hospital stay than patients without infection.[3-5]

Severe surgical stress renders the host susceptible to bacterial infections.[6,7] We previously demonstrated that burn injury markedly impairs not only interferon (IFN)-γ–mediated cellular immunity[7] but also immunoglobulin (Ig)-M–mediated humoral immunity in mice,[6] thereby decreasing survival from postburn bacterial infections. In our clinical study, the capability of peripheral blood mononuclear cells (MNCs) to produce IFN-γ significantly decreased in patients after gastrointestinal surgery.[8] However, there are few reports on the host defense response to bacterial infection in hosts with congestive heart failure.

C-reactive protein (CRP), named for its capacity to precipitate the somatic C-polysaccharide of *Streptococcus pneumoniae*, is an exquisitely sensitive marker of inflammation and tissue damage.[9] Increased serum CRP levels are associated with patient outcome in acute coronary syndromes.[10] It was recently revealed that CRP binds to Fcγ receptors I and

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Disclosures: None.

Received for publication April 29, 2009; revisions received Oct 5, 2009; accepted for publication Nov 2, 2009; available ahead of print Feb 8, 2010.

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0022-5223/$36.00

doi:10.1016/j.jtcvs.2009.11.005

Abbreviations and Acronyms

CRP = C-reactive protein
FITC = fluorescein isothiocyanate
IFN = interferon
Ig = immunoglobulin
IL = interleukin
IV = intravenously
LAD = left anterior descending
LV = left ventricular
MI = myocardial infarction
MNC = mononuclear cell
PBS = phosphate-buffered saline
TTC = triphenyltetrazolium chloride

II,[11] and thereby activates Fcγ receptor-mediated opsonization,[12] resulting in enhanced phagocytosis of microorganisms.[13] Although there are several reports that CRP-activated opsonization might exacerbate the acute phase of myocardial infarction (MI),[14] CRP-enhanced phagocytic activity may also improve the host defense response to bacterial infection, especially when the host is immunocompromised,[15] because bacterial phagocytosis is crucial to eliminate invading bacteria. The present study investigated the innate immune response to bacterial infection in mice with cardiac dysfunction induced by experimental MI.

MATERIALS AND METHODS

This study was conducted according to the guidelines of the Institutional Review Board for the Care of Animal Subjects at the National Defense Medical College, Tokorozawa, Japan.

Animals and Experimental Myocardial Infarction

Male C57BL/6 mice were studied (8 weeks old, 20 g, Japan SLC, Shizuoka, Japan). Mice were initially anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg, Abbott Laboratories, North Chicago, Ill). They were then intubated and sedated with 2% sevoflurane (Maruishi Pharm, Osaka, Japan) mixed with air and oxygen using a vaporizer (Shinano Mfg Co Ltd, Tokyo, Japan). Ventilation was performed with a rodent volume-controlled mechanical ventilator (Animal Ventilator KN-55, Natume Co Ltd, Tokyo, Japan). The heart was exposed through a left thoracotomy, and the electrocardiogram was monitored. The proximal left anterior descending (LAD) coronary artery was permanently ligated with 8-0 polypropylene to produce an infarct (MI group).[16,17] Myocardial ischemia was confirmed by regional cyanosis and ST-segment elevation. The incision was closed in layers with 5-0 polyester sutures. Sham-operated mice underwent the same procedure without ligation of the artery, but rather the suture was placed behind the LAD coronary artery (sham group) (Figure 1, *A, B*). In the MI group, 70% of the mice survived until 14 days after surgery (Figure 2, *A*).

Reagents

Escherichia coli strain B (ATCC 11303, Sigma-Aldrich Co, St Louis, MO) was grown in brain heart infusion broth (Difco Co Ltd, Detroit, Mich) and used for the experiments. Mouse recombinant interleukin (IL)-18 (Medical & Biological Laboratories, Nagoya, Japan) and mouse IgM (pp50; Chemicon International Inc, Temecula, Calif) were also used for the experiments. Synthetic CRP (174-185) was purchased from Sigma-Aldrich Co. Carboxylate microspheres (75 nm diameter; Polysciences Europe, Epipelheim, Germany) (hereafter called ''fluorescein isothiocyanate [FITC] microspheres'') were used to analyze the phagocytic activity of Kupffer cells.

Systemic *Escherichia coli* Challenge and Collection of Blood Samples

Mice were challenged intravenously (IV) with 5×10^8 colony-forming units of *E. coli* at 1, 5, or 14 days after cardiac surgery or sham operation (Figure 2, *B*). Blood samples were obtained from the retro-orbital plexus of mice at the indicated times and then stored at –80°C until assays were performed.

Interleukin-18, Immunoglobulin-M, and C-Reactive Protein Treatments

Mice were injected intraperitoneally with IL-18 (0.1 μg/0.5 mL/body) at 2 days, 4 days, and 1 hour before *E. coli* challenge, and 1, 3, and 5 days after *E. coli* challenge, which was performed 5 days after surgery (Figure 2, *C*). Mice were also subjected to surgery and then injected IV with IgM (330 μg/0.5 mL/body) 1 hour before *E. coli* challenge and 2 days after challenge, or injected IV with CRP (500 μg/0.5 mL/body) 1 hour before *E. coli* challenge (Figure 2, *C*). Sham treatment was performed by injection with phosphate-buffered saline (PBS) (0.5 mL) in the same manner as the IL-18, IgM, and CRP treatment.

Blood Culture and Histologic Examinations

Blood culture and histologic examinations of the heart, lung, liver, and kidney were performed as described.[6,7,15]

Isolation of Mononuclear Cells and Cell Cultures

MNCs were obtained from the liver, spleen, and femurs as previously described.[6,7,18] After counting cells, 5×10^5 of liver, spleen, or bone marrow MNCs in 200 μL 10% fetal bovine serum-Roswell Park Memorial Institute 1640 medium were cultured in 96-well flat-bottom plates in 5% CO_2 at 37°C for 24 hours.[6,7,18]

Measurements of Cytokine and Immunoglobulin-M Levels in Culture Supernatants and Sera

Cytokine levels of the sera or the culture supernatants were measured using commercially available enzyme-linked immunosorbent assay kits (IL-18, MBL, Nagoya, Japan; others, Endogen, Woburn, Mass). IgM levels of sera or culture supernatants were measured using an enzyme-linked immunosorbent assay quantification kit (Bethyl Laboratories Inc, Montgomery, Tex).

Determination of Microsphere Phagocytic Activity of Kupffer Cells

Mice were injected IV with FITC microspheres (20 μL/0.5 mL/body). After 20 minutes, they were euthanized to remove the livers. The liver MNCs, including Kupffer cells, were stained with phycoerythrin-conjugated anti-mouse F4/80 Ab (eBioscience, San Diego, Calif). Thereafter, phagocytosis of FITC microspheres by $F4/80^+$ hepatic macrophages, namely, Kupffer cells, was analyzed using the EPICS XL (Beckman Coulter Inc, Miami, Fla). Because FITC fluorescence intensity is dependent on the number of ingested microspheres, the peaks of the histogram correspond to Kupffer cells that contained no ingested microspheres (peak 0) and 1 (peak 1), 2 (peak 2), and more (peak ≥ 3) microspheres from left to right, respectively.

Assessment of Myocardial Infarction

Echocardiography. Two-dimensionally guided M-mode recordings were obtained from the short-axis view at the level of the papillary muscles using an HD11XE ultrasound system and a 15-MHz linear-array transducer (Royal Philips Electronics, Eindhoven, The Netherlands). LV end-systolic and end-diastolic dimensions, as well as systolic and diastolic wall thickness,

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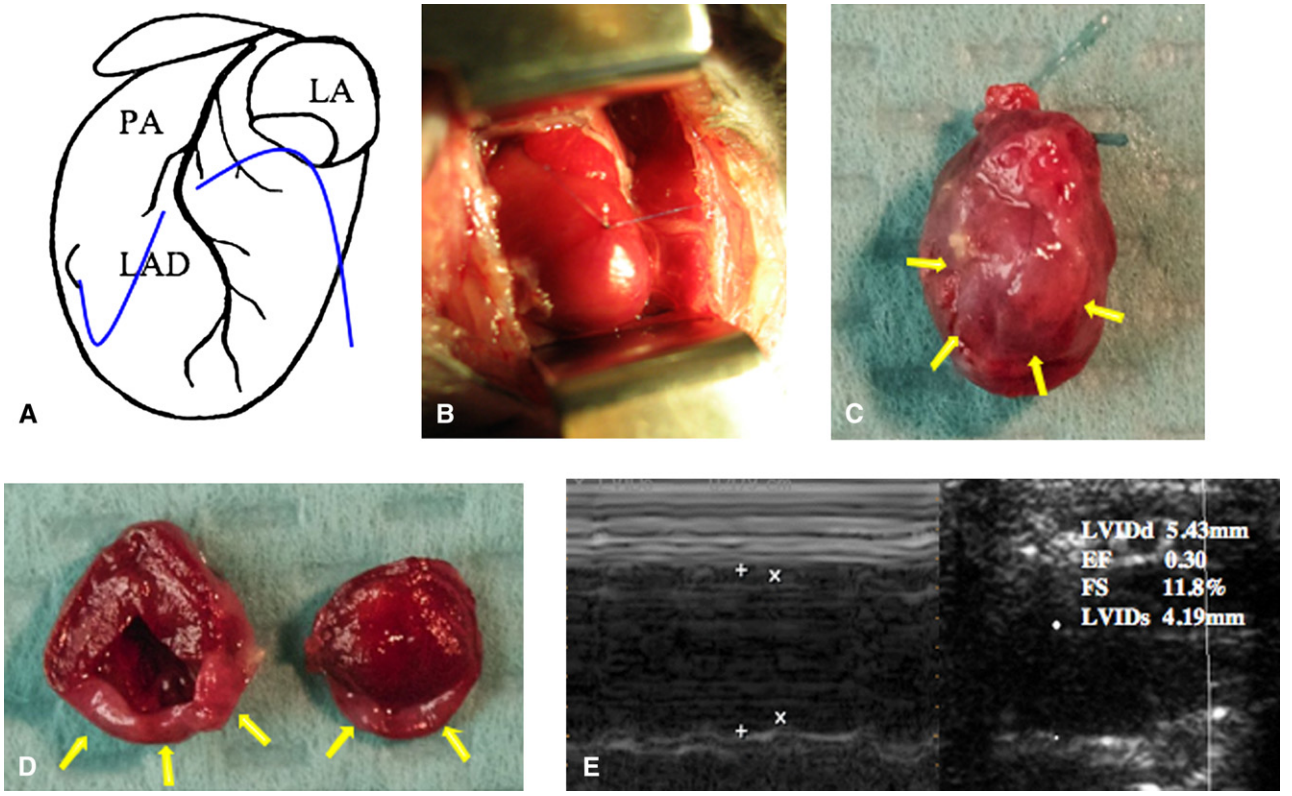

FIGURE 1. A, LAD ligation. B, Open-chest mouse with ligation of the LAD. C, Heart of a mouse with MI. D, Transverse sections of the heart. E, Echocardiography of a mouse with MI. Data shown are representative of 10 mice with similar results. *PA,* Pulmonary artery; *LA,* left atrium; *LAD,* left anterior descending; *LVIDd,* left ventricular internal dimension diastole; *EF,* ejection fraction; *FS,* fractional shortening; *LVIDs,* left ventricular internal dimension systole.

were measured from the M-mode tracings using the leading-edge convention of the American Society of Echocardiography. For each M-mode measurement, at least 3 consecutive cardiac cycles were sampled. LV mass and ejection fraction were calculated from the short-axis wall thickness, and chamber dimension was measured assuming a spherical LV geometry.[19,20]

Postmortem examination. Animals were reanesthetized, and the chest was reopened with the same procedure as described above. After euthanasia, the heart was removed, and the left ventricle was sliced transversely. The slices were then incubated for 10 minutes in a 1% solution of buffered triphenyltetrazolium chloride (TTC), immersed in 10% buffered formalin phosphate, and photographed. The infarcted zone was not stained with TTC, whereas the noninfarcted zone was stained brick red with TTC.

Statistical Analysis

The data are presented as the mean ± standard error. Statistical analyses were performed using a Mac computer (Apple, Cupertino, Calif) and the Stat View 4.02 J software package (Abacus Concepts, Berkeley, Calif). Survivals were compared using the Wilcoxon rank test, and other statistical evaluations were compared using the standard 1-way analysis of variance followed by the Bonferroni post hoc test. Pearson's correlation coefficient was used to examine the correlation between ejection fraction and survival.

RESULTS

Cardiac Dysfunction Induced by Left Anterior Descending Ligation

Five days after experimental MI, the left ventricle was markedly enlarged, and there was increased sphericity (Figure 1 *C, arrows* indicate the MI area) and wall thinning (Figure 1, *D, arrows*). The LV-infarcted area was 29.7% ± 4.4% (n = 5), as assessed by TTC staining 1 day after LAD ligation, and not significantly changed until 14 days. Mice with MI also showed LV chamber dilatation and poor wall motion on echocardiography (Figure 1, *E*), suggesting severe cardiac dysfunction. Cardiac function, including the ejection fraction of mice with MI, decreased markedly after LAD ligation (Figure E1, *A–F*).

Cardiac Dysfunction Decreases Mouse Survival From *Escherichia coli* Challenge 5 Days After Surgery

The MI and sham groups showed a similar survival from *E. coli* challenge at 1 day after surgery, which was significantly lower than the survival of the nontreated group (Figure 3, *A*). However, the MI group showed a significantly lower survival from *E. coli* challenge at 5 days after surgery compared with the sham and nontreated groups (Figure 3, *A*). Both the MI and sham groups showed survival similar to the nontreated group at 14 days after surgery (Figure 3, *A*). The relationship between cardiac function (ejection fraction) and survival from *E. coli* infection was examined. The ejection fraction correlated strongly with their survival from infection (Figure 3, *B*). Mice with MI 5 days after surgery showed significantly higher bacterial counts in the blood after *E. coli* infection than those of the sham and nontreated mice (Figure 3, *C*),

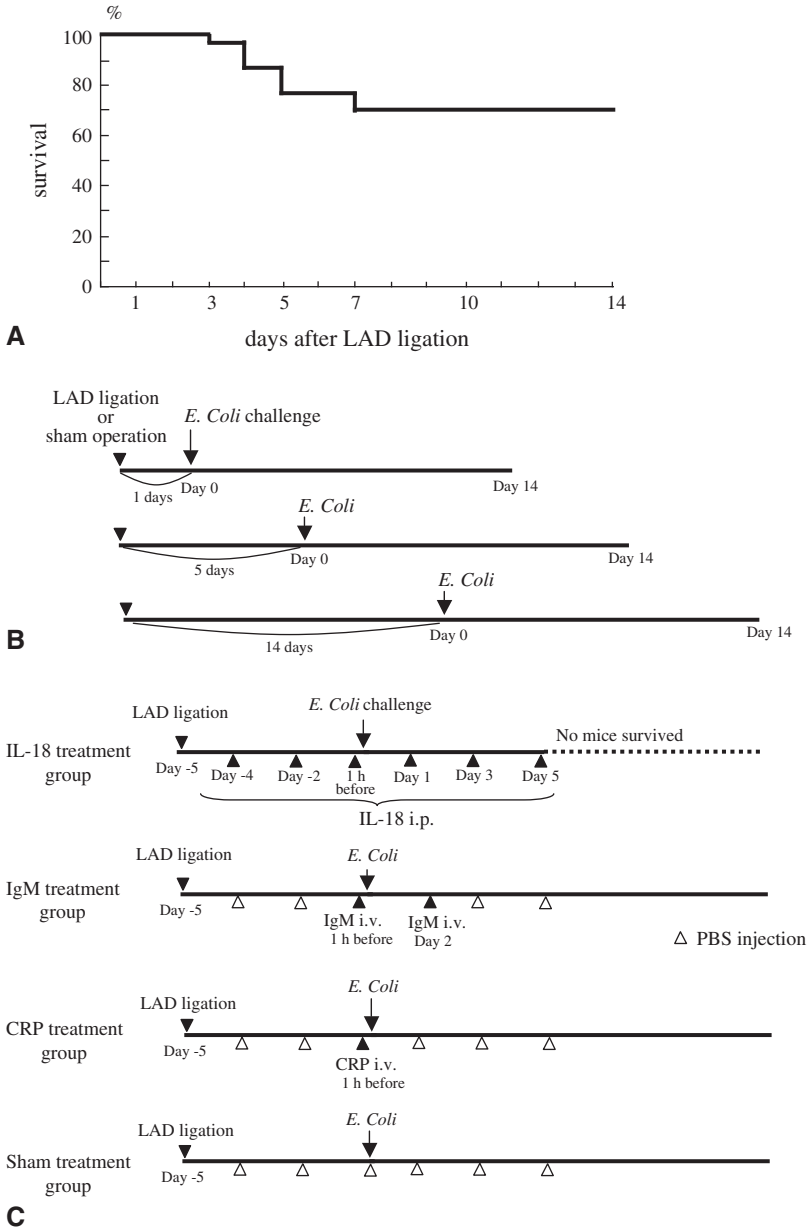

FIGURE 2. A, Survival of mice with MI after LAD ligation (without *Escherichia coli* challenge). B, Study designs of the *E. coli* challenge model in mice at 1, 5, and 14 days after LAD ligation or sham operation. C, Study designs of treatment with IL-18, IgM, or CRP for mice challenged with *E. coli* 5 days after LAD ligation. *LAD,* Left anterior descending; *IL,* interleukin; *Ig,* immunoglobulin; *PBS,* phosphate-buffered saline; *CRP,* C-reactive protein; *i.p.,* intraperitoneally; *i.v.,* intravenously.

and showed pathologic findings in various organs caused by bacterial infection (Figure E2). These findings suggest that the inhibitory effect of cardiac dysfunction on the host defense response to infection is significant at 5 days after surgery.

Cardiac Dysfunction Suppresses Serum Elevation of Proinflammatory Cytokines After *Escherichia coli* Challenge in Mice 5 Days After Surgery

The MI group (5 days after surgery) showed significantly lower peaks of serum TNF at 1 hour, IL-12 at 3 hours, and IFN-γ at 6 hours after *E. coli* challenge than the other groups (Figure 4, *A, C,* Figure E3, *A*). Although the MI group also showed a significantly lower peak of IL-18 at 12 hours than the other groups, the mice with MI showed a significant increase in IL-18 levels at 5 days (Figure 3, *B*). However, there were no significant differences in these cytokine responses to *E. coli* challenge between the surviving and deceased mice with MI (data not shown). No significant differences in the change in serum IgM levels were observed among the 3 groups (Figure E3, *B*).

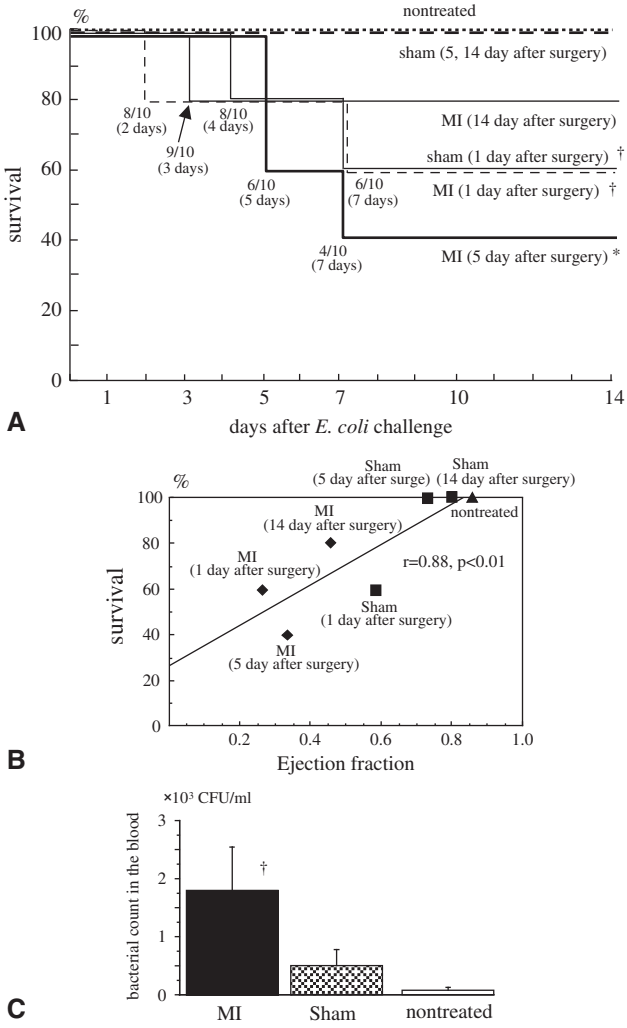

FIGURE 3. A, Survival after *E. coli* challenge in the MI, sham, and nontreated groups 1, 5, and 14 days after surgery. Survival was determined using 10 mice in each group. *$P < .01$ vs sham (5 days after surgery) and nontreated. †$P < .05$ vs nontreated. B, Relationship between the ejection fraction and mouse survival after *E. coli* challenge. Immediately before *E. coli* challenge, the ejection fractions were evaluated. Then, the relationships of the mean values of the ejection fractions and survivals at 14 days after *E. coli* challenge were examined. Ejection fraction and survival were determined using 10 mice in each group. C, Blood cultures 24hours after *E. coli* challenge in the MI, sham, and nontreated groups. Mice were challenged IV with *E. coli* 5 days after surgery in each group. After 24hours, blood samples were obtained from the mice and cultured. Data represent mean ± standard error (SE) for 5 mice in each group. †$P < .05$ vs other groups. *MI,* Myocardial infarction.

Cardiac Dysfunction Suppresses Production of Tumor Necrosis Factor, Interferon-γ, and Immunoglobulin-M in Mouse Liver Mononuclear Cells After *Escherichia coli* Challenge

Mice were challenged with *E. coli* at 5 days after surgery in the MI and sham groups. Nontreated mice also received *E. coli* challenge (nontreated group). Thereafter, liver, spleen, and bone marrow MNCs were obtained from mice 2 hours after *E. coli* challenge and cultured for 24 hours. The MI group showed significantly lower production of TNF and IFN-γ from liver MNCs than the sham group, but higher production of these cytokines from spleen MNCs (Figure 4, *D, E*). Other mice were also similarly challenged with *E. coli* at 5 days after surgery. Because serum IgM peaked at 5 days after bacterial challenge,[6] liver, spleen, and bone marrow MNCs were obtained from mice at 4 days and then cultured for 24 hours. The MI group showed significantly lower IgM production from liver MNCs than the sham group, although no difference in IgM production by spleen or bone marrow MNCs was observed among the 3 groups (Figure 4, *F*).

Cardiac Dysfunction Suppresses the Phagocytic Activity of Kupffer Cell in Mice 5 Days After Surgery

The MI group had a lower proportion of Kupffer cells that showed microsphere phagocytosis (peaks 2 and ≥ 3), but

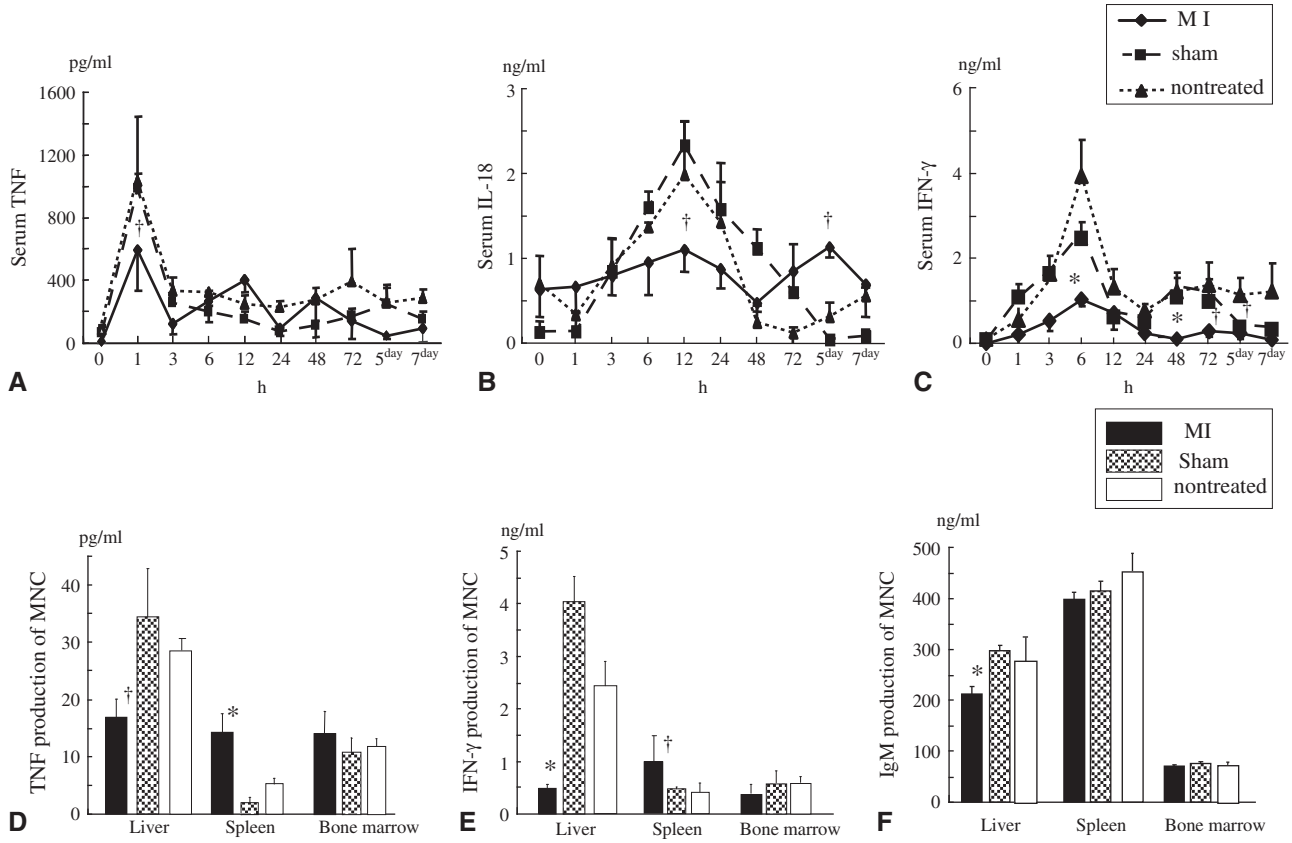

FIGURE 4. A–C, Changes in serum cytokine levels after *E. coli* challenge in MI, sham, and nontreated groups. Mice were challenged IV with *E. coli* 5 days after surgery. Nontreated mice were also challenged with *E. coli*. Serum TNF (A), IL-18 (B), and IFN-γ (C) levels were measured at the indicated times. Data represent mean ± SE for 7 mice in each group. *$P < .01$. †$P < .05$ vs other groups. D–F, Cytokine and IgM production in mouse MNC after *E. coli* challenge. *E. coli* was similarly challenged IV into the mice 5 days after surgery. The liver, spleen, and bone marrow MNCs were obtained 2hours after *E. coli* challenge and cultured for 24hours to measure TNF (D) and IFN-γ (E) production. F, MNCs were also obtained 4 days after *E. coli* challenge and cultured for 24hours to measure IgM production. Data represent mean ± SE from 3 to 4 mice in 3 independent experiments. *$P < .01$. †$P < .05$ vs sham. *MI,* Myocardial infarction; *TNF,* tumor necrosis factor; *IL,* interleukin; *IFN,* interferon; *MNC,* mononuclear cell; *Ig,* immunoglobulin.

a higher proportion of Kupffer cells that phagocytosed no microspheres (non-phagocytic) at 5 days after surgery compared with the sham group (Figure 5, *A*). Both the MI and sham groups had a significantly lower proportion of Kupffer cells that showed potent phagocytic activity (peak ≥ 3) at 1 day after surgery compared with the nontreated group (Figure 5, *B*). However, the MI group had a significantly lower proportion of phagocytosing Kupffer cells at 5 days than the sham and nontreated groups (Figure 5, *B*). Both the MI and sham groups had similar proportions of phagocytosing Kupffer cells compared with the nontreated group at 14 days (Figure 5, *B*). We next compared the peaks of serum TNF at 1 hour and IFN-γ at 6 hours after *E. coli* challenge between the MI and sham mice. Mice with MI at 5 days after surgery showed lower serum peaks of TNF and IFN-γ than sham mice, but no significant differences were observed on days 1 and 14 (Figure E4, *A, B*). These peaks of cytokines in both mice were remarkably suppressed on day 1 and restored on day 14 (Figure E4, *A, B*). Suppression of the serum IgM response to *E. coli* challenge was not observed in mice with MI on days 1, 5, or 14 (data not shown).

Neither Interleukin-18 nor Immunoglobulin-M Treatment Improves Survival After *Escherichia coli* Challenge in Mice With Myocardial Infarction

We previously reported that IL-18 treatment was effective against *E. coli* or *Pseudomonas aeruginosa* infection in burn-injured mice by increasing IFN-γ or IgM production, respectively.[6,7] Therefore, mice with MI were treated with multiple IL-18 or PBS injections on alternate days before and after *E. coli* challenge, which was performed 5 days after MI induction. Unexpectedly, IL-18 treatment further reduced the survival of mice with MI after *E. coli* challenge (Figure 5, *C*). IL-18 treatment remarkably increased serum IFN-γ levels after *E. coli* challenge in mice, even after experimental MI (data not shown). We next treated mice with MI with IgM injections immediately before and 2 days after *E. coli* challenge. However, IgM treatment

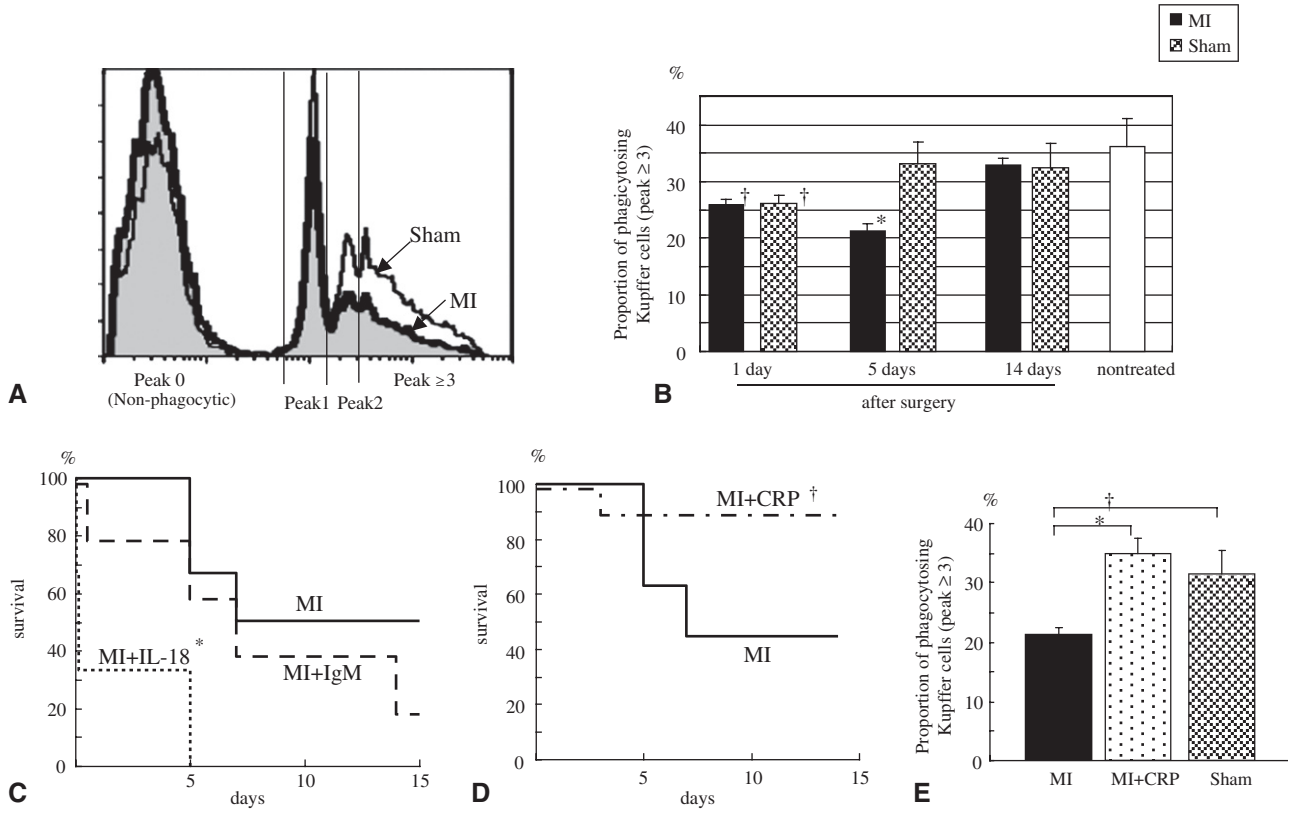

FIGURE 5. Microsphere phagocytosis by Kupffer cells was analyzed in the mice as described in the ''Materials and Methods'' section. A, Representative data of phagocytosis by Kupffer cells of mice with MI and sham mice 5 days after surgery are shown. B, Proportion of phagocytosing Kupffer cells in peak ≥ 3 in each group at 1, 5, and 14 days after surgery. Data represent mean ± SE from 3 to 4 mice in 3 independent experiments. *$P < .01$ vs all other groups. †$P < .01$ vs nontreated group. C, Survival after *E. coli* challenge in mice with MI treated with IL-18 or IgM. Mice with MI were treated with multiple IL-18 injections, IgM injections, or control PBS injections (see the ''Materials and Methods'' section for the IL-18 or IgM treatment schedule). Then, mice with MI were challenged IV with *E. coli* 5 days after MI induction. D, Survival after *E. coli* challenge in mice with MI treated with CRP. Mice with MI were injected IV with CRP or PBS at 5 days after MI induction. After 1hour, they were challenged IV with *E. coli*. E, Phagocytic activity of Kupffer cells in mice with MI treated with CRP. One hour after CRP/PBS treatment, MI or sham mice were injected IV with FITC microspheres. Thereafter, phagocytosis by Kupffer cells was analyzed. C, D, N = 10 in each group. E, Data represent mean ± SE from 3 to 4 mice in 3 independent experiments. *$P < .01$. †$P < .05$ vs others. *MI,* Myocardial infarction; *IL,* interleukin; *Ig,* immunoglobulin; *CRP,* C-reactive protein.

did not improve the survival after *E. coli* challenge in mice with MI (Figure 5, *C*).

C-Reactive Protein Treatment Improves Survival After *Escherichia coli* Challenge in Mice With Myocardial Infarction

We attempted to stimulate the phagocytic activity of Kupffer cells using CRP injection in mice with MI. Mice were injected IV with CRP or PBS at 5 days after MI induction. After 1 hour, they were challenged with *E. coli*. CRP treatment significantly improved survival after *E. coli* challenge (Figure 5, *D*). CRP treatment significantly augmented phagocytic activity by Kupffer cells in mice with MI (Figure 5, *E*). We recently reported that CRP treatment not only enhances the phagocytic activity of Kupffer cells but also suppresses the elevations of serum TNF and IFN-γ after *E. coli* challenge in burn-injured mice.[15] In line with this, CRP treatment further suppressed the elevation of serum TNF at 1 hour after *E. coli* challenge and IFN-γ at 6 hours in the mice with MI (data not shown), suggesting that restoration of phagocytic activity by CRP treatment is crucial for mice with MI to survive from bacterial infections.

DISCUSSION

We previously demonstrated that liver MNCs, especially natural killer/natural killer T cells, are major IFN-γ producers in response to the stress of burn injury. Furthermore, they become unresponsive/anergic to bacterial infections at 5 to 10 days after burn injury because they cannot efficiently produce IFN-γ.[7] IFN-γ–mediated cellular immunity also might be suppressed in the host after massive hemorrhage, resulting in susceptibility to bacterial infection/sepsis.[21] IgM is an important defense mechanism against bacteria, which takes effect before the IgG-mediated defense

mechanism begins.[22] Burn injury also reduces IgM production in the host and thereby renders the host susceptible to bacterial infection.[6] Responses of proinflammatory cytokines, such as TNF and IFN-γ, to *E. coli* challenge were significantly suppressed in mice with MI at 5 days after surgery compared with those in sham mice, whereas these responses were similarly impaired at 1 day after surgery but restored at 14 days in both MI and sham mice. Mice with MI also showed a significantly poor survival from infection at 5 days compared with sham mice, whereas both MI and sham mice showed similar poor survival at 1 day and good prognoses at 14 days. Thus, changes in the proinflammatory cytokine response may coincide with changes in mouse survival from infection. Mice with MI also had decreased IgM production by liver MNCs. Cardiac dysfunction induced by MI and other surgical stresses might impair cellular and humoral immunities against bacterial infection.

Impairment of the ejection fraction in mice strongly correlated with susceptibility to *E. coli* infection. However, the mice with MI 5 days after LAD ligation showed lower survival from infection compared with the mice with MI 1 day after surgery, although the former mice with MI (5 days after surgery) showed a little higher ejection fraction than the latter mice with MI (1 day after surgery). The duration of cardiac dysfunction (5 days) in mice with MI may affect their susceptibility to bacterial infection. However, enhancement of cellular or humoral immunity by IL-18 or IgM treatment did not improve survival from infection in mice with MI, suggesting that congestive heart failure after cardiac dysfunction induces a profound and multifactorial immune suppression.

Cardiac dysfunction induced by MI also markedly suppressed the phagocytic activity of Kupffer cells in mice at 5 days after surgery. The phagocytic activity of Kupffer cells was depressed in both MI and sham-operated mice compared with nontreated mice at 1 day after surgery, but this depressed activity was recovered at 14 days after surgery. These functional changes in Kupffer cells coincided with changes in mouse survival after the *E. coli* challenge. Several investigators have demonstrated that the phagocytic activity of Kupffer cells was inhibited after hemorrhage, endotoxin shock, and thermal injury.[23] Because liver-resident macrophages, Kupffer cells, are known to represent 80% to 90% of the resident macrophages in the body, more than 70% of the bacteria that enter the bloodstream accumulate in the liver and are trapped by Kupffer cells.[24] Improvement/restoration of the phagocytic activity of Kupffer cells might be crucial to protect mice with MI against bacterial infection.

Enhancement of the phagocytic activity of Kupffer cells by CRP treatment significantly improved survival after infection in mice with MI. The up-regulation of phagocytic activity by Kupffer cells may be more important to increase the survival of mice with MI from infection than the up-regulation of the cellular immune response by IL-18 or the humoral immune response by IgM. CRP treatment stimulates macrophages to phagocytose several organisms, such as *Streptococcus pneumoniae*.[11,13] The cause of the Kupffer cell dysfunction resulting from the cardiac dysfunction observed in this study remains to be clarified. Circulatory collapse from hemorrhage, trauma, or congestive heart failure after cardiac dysfunction might lead to a decrease in liver blood flow.[25] This deceased blood flow may suppress the function of liver MNCs, including Kupffer cells, resulting in impairment of bacterial clearance and host defense.[25] Unlike liver MNCs, spleen MNCs increased TNF and IFN-γ production and did not decrease IgM production after *E. coli* challenge in mice with MI. Congestive heart failure after cardiac dysfunction may directly impair the function of liver MNCs compared with spleen MNCs. Because of the anatomic proximity of the liver to the heart, the liver may be more vulnerable than the spleen to any condition (eg, congestive heart failure) that elevates right atrial pressure.

Several investigators have reported that CRP increases the size of MI in rats subjected to coronary artery ligation.[14] The possibility is raised that because CRP potently activates macrophages, activated macrophages in the heart might augment phagocytosis of damaged myocardial tissue. Consistent with this hypothesis, CRP treatment immediately after experimental MI exacerbated infarct size, thereby increasing mortality (CRP, 75% vs control, 30%; $P < .01$). Because macrophages in the heart may be activated substantially by ischemic stress in the acute phase of MI, CRP stimulation in this phase may induce further or excessive activation of these macrophages, increasing the amount of myocardial necrosis. However, macrophages such as Kupffer cells might become unresponsive/anergic to infection in the next phase, namely, the phase of congestive heart failure after cardiac dysfunction ($\sim$5 days after MI induction). The delayed development of this unresponsive/anergic condition in the host after MI induction seems to be similar to the condition that develops after burn injury.[6,7] Restoration of anergic cell function seems to be important for host defense against bacterial infection after MI as well as after burn injury.[7]

IL-18 treatment reduced survival after *E. coli* challenge in mice with MI. IL-18 treatment greatly enhanced IFN-γ production after bacterial challenge in these mice (data not shown). This IL-18–induced exaggerated IFN-γ production may cause multiorgan injury, and IFN-γ may also further activate macrophages in the heart, leading to higher mortality. IL-18 treatment also increases mortality in normal mice after *E. coli* challenge because of IL-18–enhanced IFN-γ production, despite the fact that IL-18 saved burn-injured mice from *E. coli* infection.[7]

CONCLUSIONS

Our findings provide new insight into the enhanced susceptibility to infection in patients with congestive heart failure after cardiac dysfunction from the viewpoint of the host

defense immune system. Elucidation of mechanisms for altered host defense against perioperative infection in heart failure/low cardiac output may help in the development of new clinical strategies for the prevention and treatment of infections, improving patient outcomes after cardiac surgery.

References

1. Trachiotis GD, Weintraub WS, Johnston TS, Jones EL, Guyton RA, Craver JM. Coronary artery bypass grafting in patients with advanced left ventricular dysfunction. *Ann Thorac Surg*. 1998;66:1632-9.
2. Reddy SL, Grayson AD, Smith G, Warwick R, Chalmers JA. Methicillin resistant Staphylococcus aureus infections following cardiac surgery: incidence, impact and identifying adverse outcome traits. *Eur J Cardiothorac Surg*. 2007;32:113-7.
3. Fowler VG Jr, O'Brien SM, Muhlbaier LH, Corey GR, Ferguson TB, Peterson ED. Clinical predictors of major infections after cardiac surgery. *Circulation*. 2005;112(9 Suppl):I358-65.
4. De Santo LS, Bancone C, Santarpino G, Romano G, De Feo M, Scardone M, et al. Microbiologically documented nosocomial infections after cardiac surgery: an 18-month prospective tertiary care centre report. *Eur J Cardiothorac Surg*. 2008;33:666-72.
5. Falagas ME, Rosmarakis ES, Rellos K, Michalopoulos A, Samonis G, Prapas SN. Microbiologically documented nosocomial infections after coronary artery bypass surgery without cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. 2006;132:481-90.
6. Kinoshita M, Shinomiya N, Ono S, Tsujimoto H, Kawabata T, Matsumoto A, et al. Restoration of natural IgM production from liver B cells by exogenous IL-18 improves the survival of burn-injured mice infected with Pseudomonas aeruginosa. *J Immunol*. 2006;177:4627-35.
7. Kinoshita M, Seki S, Ono S, Shinomiya N, Hiraide H. Paradoxical effect of IL-18 therapy on the severe and mild Escherichia coli infections in burn-injured mice. *Ann Surg*. 2004;240:313-20.
8. Hiraki S, Ono S, Kinoshita M, Tsujimoto H, Seki S, Mochizuki H. Interleukin-18 restores immune suppression in patients with nonseptic surgery, but not with sepsis. *Am J Surg*. 2007;193:676-80.
9. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003;111:1805-12.
10. Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in ''active'' coronary artery disease. *Am J Cardiol*. 1990;65:168-72.
11. Stein MP, Edberg JC, Kimberly RP, Mangan EK, Bharadwaj D, Mold C, et al. C-reactive protein binding to FcgammaRIIa on human monocytes and neutrophils is allele-specific. *J Clin Invest*. 2000;105:369-76.
12. Bodman-Smith KB, Gregory RE, Harrison PT, Raynes JG. FcgammaRIIa expression with FcgammaRI results in C-reactive protein- and IgG-mediated phagocytosis. *J Leukoc Biol*. 2004;75:1029-35.
13. Mold C, Rodriguez W, Rodic-Polic B, Du Clos TW. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. *J Immunol*. 2002;169:7019-25.
14. Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, et al. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med*. 1999;190:1733-40.
15. Inatsu A, Kinoshita M, Nakashima H, Shimizu J, Saitoh D, Tamai S, et al. Novel mechanism of C-reactive protein for enhancing mouse liver innate immunity. *Hepatology*. 2009;49:2044-54.
16. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, et al. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med*. 2006;12:459-65.
17. Michael LH, Entman ML, Hartley CJ, Youker KA, Zhu J, Hall SR, et al. Myocardial ischemia and reperfusion: a murine model. *Am J Physiol*. 1995;269(6 Pt 2): H2147-54.
18. Ami K, Kinoshita M, Yamauchi A, Nishikage T, Habu Y, Shinomiya N, et al. IFN-gamma production from liver mononuclear cells of mice in burn injury as well as in postburn bacterial infection models and the therapeutic effect of IL-18. *J Immunol*. 2002;169:4437-42.
19. Boluyt MO, Converso K, Hwang HS, Mikkor A, Russell MW. Echocardiographic assessment of age-associated changes in systolic and diastolic function of the female F344 rat heart. *J Appl Physiol*. 2004;96:822-8.
20. Takuma S, Suehiro K, Cardinale C, Hozumi T, Yano H, Shimizu J, et al. Anesthetic inhibition in ischemic and nonischemic murine heart: comparison with conscious echocardiographic approach. *Am J Physiol Heart Circ Physiol*. 2001;280: H2364-70.
21. Zellweger R, Ayala A, DeMaso CM, Chaudry IH. Trauma-hemorrhage causes prolonged depression in cellular immunity. *Shock*. 1995;4:149-53.
22. Kinoshita M, Kuranaga N, Matsumoto A, Ono S, Shinomiya N, Hiraide H, et al. Multiple interleukin-18 injections promote both mouse Th1 and Th2 responses after sublethal Escherichia coli infection. *Clin Exp Immunol*. 2006;143:41-9.
23. Vollmar B, Bauer C, Menger MD. n-3 Polyunsaturated fatty acid-enriched diet does not protect from liver injury but attenuates mortality rate in a rat model of systemic endotoxemia. *Crit Care Med*. 2002;30:1091-8.
24. Seki S, Habu Y, Kawamura T, Takeda K, Dobashi H, Ohkawa T, et al. The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells, natural killer (NK) cells and NK1.1 Ag+T cells in T helper 1 immune responses. *Immunol Rev*. 2000;174:35-46.
25. Coleman DL. Regulation of macrophage phagocytosis. *Eur J Clin Microbiol*. 1986;5:1-5.

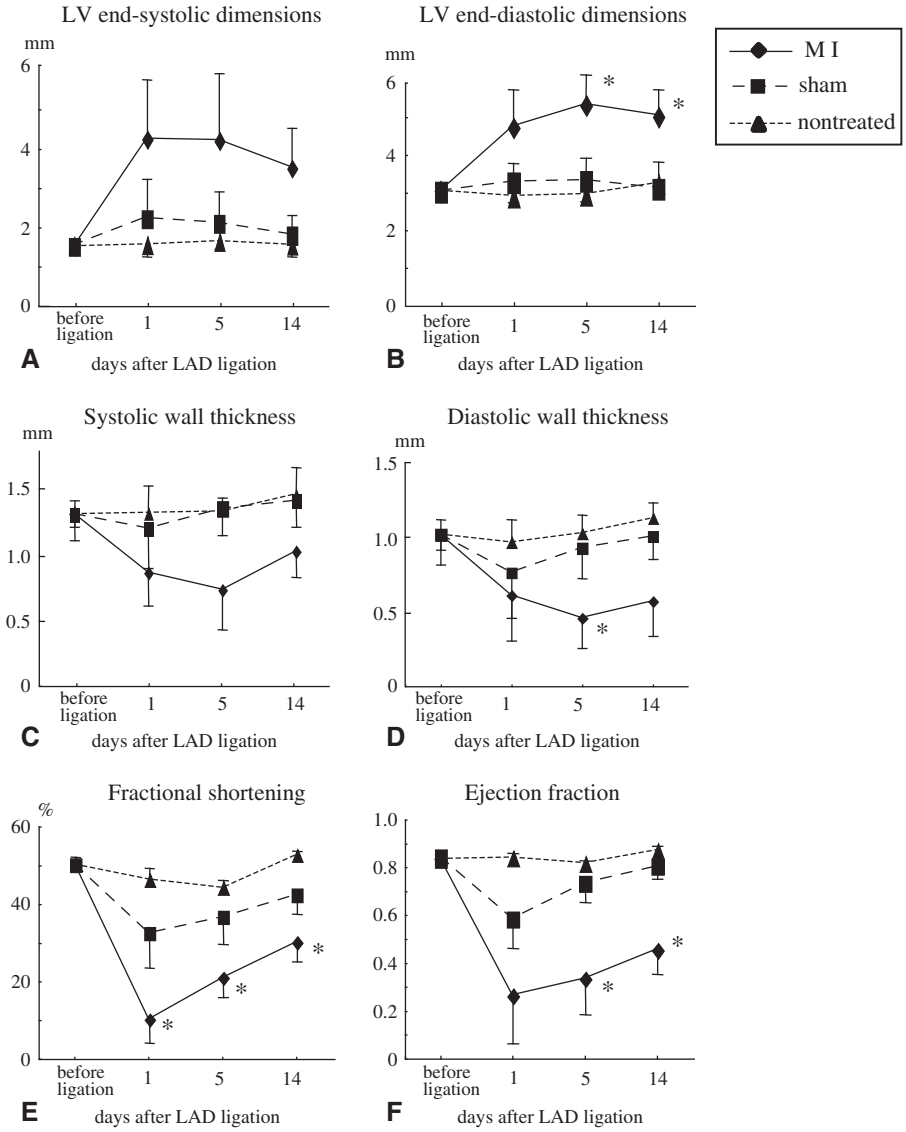

FIGURE E1. Echocardiographic assessment of cardiac function after LAD ligation. The changes of LV end-systolic dimensions (A), LV end-diastolic dimensions (B), systolic wall thickness (C), diastolic wall thickness (D), fractional shortening (E), and ejection fraction (F) were evaluated after surgery in the MI, sham, and nontreated groups. Data represent mean ± SE for 5 mice in each group. *$P < .05$ vs other groups. *LV,* Left ventricular; *MI,* myocardial infarction; *LAD,* left anterior descending.

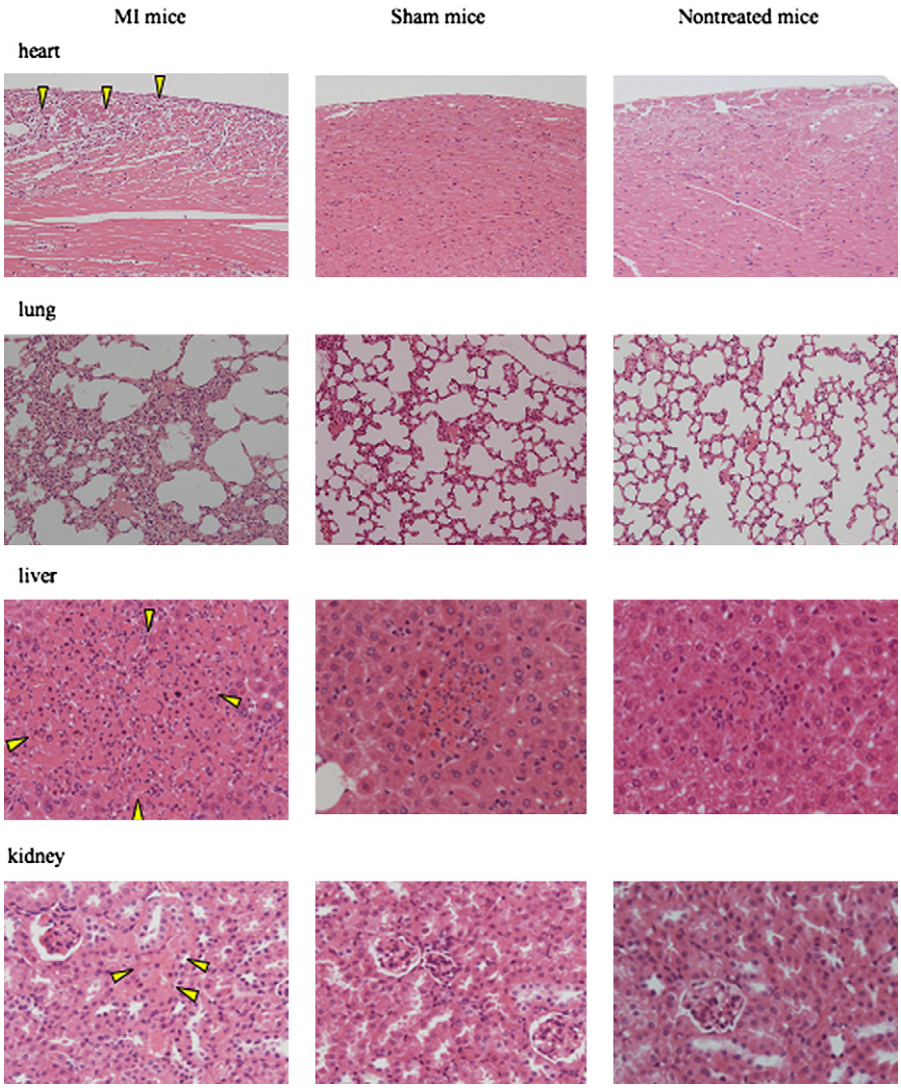

FIGURE E2. Pathologic findings from the MI, sham, and nontreated mice 24hours after *E. coli* challenge. MI and sham mice were challenged IV with *E. coli* 5 days after surgery. Nontreated mice were also challenged IV with *E. coli*. Mice with MI showed obvious myocardial injury caused by infarction (*arrows*) and showed alveolar edema and septum thickness in the lung, coagulation necrosis in the liver (*arrows*), and acute tubular degeneration in the kidney (*arrows*). Data shown are representative of 5 mice with similar results. Specimens from the heart, lung, liver, and kidney (×200 magnification) are shown (hematoxylin-eosin staining). *MI,* Myocardial infarction.

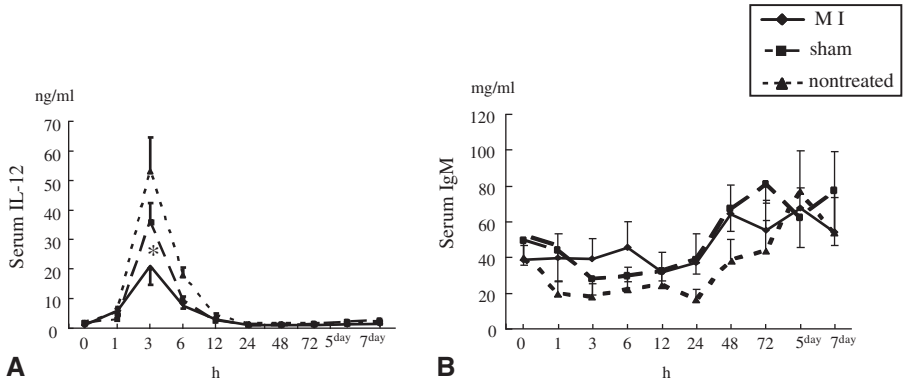

FIGURE E3. Changes in serum IL-12 and IgM levels after *E. coli* challenge in MI, sham, and nontreated mice. Mice were challenged IV with *E. coli* 5 days after cardiac/sham surgery. Nontreated mice were also challenged IV with *E. coli*. Serum levels of IL-12 (A) and IgM (B) were measured at the indicated times. Data represent mean ± SE for 7 mice in each group. *$P < .05$ versus others. *MI,* Myocardial infarction; *IL,* interleukin; *Ig,* immunoglobulin.

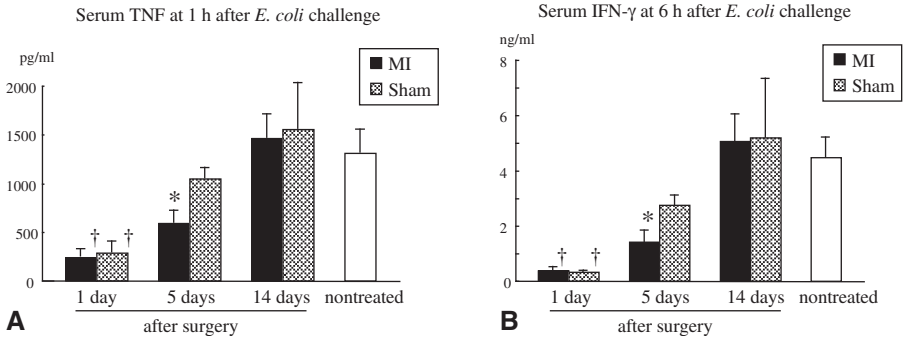

FIGURE E4. Serum peaks of TNF (A) and IFN-γ (B) after *E. coli* challenge in MI and sham mice on days 1, 5, and 14 after surgery. MI and sham mice were challenged IV with *E. coli* 1, 5, and 14 days after surgery. Nontreated mice were also challenged with *E. coli*. Serum TNF and IFN-γ levels were determined 1hour and 6hours after *E. coli* challenge, respectively. Data represent mean ± SE from 7 mice in each group. *$P < .01$ vs sham (5 days) and nontreated. †$P <$ .01 vs nontreated. *TNF,* Tumor necrosis factor; *IFN,* interferon; *MI,* myocardial infarction.